

# Investigation of the contribution of concentrated growth factor (CGF) and processed lipoaspirate (PLA) to wound healing in diabetic rats

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## ABSTRACT

**Aim:** The aim of the study is to show the effectiveness of concentrated growth factor (CGF) and processed lipoaspirate (PLA) in wound healing in diabetic rats.

**Materyal and Method:** A total of 30 rats were used in the study. It was divided into 3 groups as concentrated growth factor, processed lipoaspirate and control group. The rats were made diabetic using Streptozotocin IP. A 5mm diameter wound was created on one of the hind legs of all rats by using a punch. Concentrated growth factor and processed lipoaspirate were applied to the lesions. Daily wound size and wound condition were recorded on days 3, 5 and 10. At the end of the study, blood samples were taken for TNF- $\alpha$ , TGF- $\beta$ , IL-1, PDGF, FGF and VEGF measurements before the rats were sacrificed.

**Results:** The mean wound diameters measured on the 3<sup>rd</sup> day in the study were 4.6 $\pm$ 0.06 mm in the control group, 4.1 $\pm$ 0.05 mm in the concentrated growth factor group, and 4.4 $\pm$ 0.07 mm in the processed lipoaspirate group. The wound diameters measured on the 5<sup>th</sup> day were 3.1 $\pm$ 0.04 mm in the control group, 1.6 $\pm$ 0.05 mm in the concentrated growth factor group and 2.7 $\pm$ 0.06 mm in the processed lipoaspirate group ( $p < 0.01$ ). The mean closure time of wounds was 5.3 $\pm$ 1.1 days in the concentrated growth factor group, 7.1 $\pm$ 1.4 days in the processed lipoaspirate group, and 9.4 $\pm$ 0.5 days in the control group. All of the wounds were healed in all groups on the 10<sup>th</sup> day. This improvement rate in the concentrated growth factor group was statistically significant compared to the other two groups ( $p < 0.01$ ). Concentrated growth factor and PLA increased the speed of wound healing in diabetic rats. Inflammatory marker levels (TNF- $\alpha$ , TGF- $\beta$ , IL-1, PDGF, FGF, VEGF) obtained from blood samples were higher than normal in all rats and there was no significant difference between the groups ( $p > 0.05$ ).

**Conclusion:** In this study, it was shown that concentrated growth factor application was more effective than processed lipoaspirate application in wound healing in diabetic rats.

**Keywords:** CGF, PLA, diabetic rat, ulcer

## INTRODUCTION

Wound is the disruption of the tissue integrity of the skin or mucosa for many different reasons such as abrasions, cuts, stings, bruises, burns, venous ulcers, surgical incisions and diabetic ulcers. Damaged tissue repair begins with hemostasis. Then the inflammatory period begins and is completed in 24-48 hours. It is completed in 3 stages as proliferative and maturation stages (1,2).

When the vessel wall is damaged, thrombocytes contact the collagen in the opened vessel wall and form a temporary clot and hemostasis is achieved. Inflammatory cells migrate towards the wound area and begin to remove apoptotic cells and bacteria from the wound area. Cytokines are released immediately after tissue damage in the inflammatory period. Cytokines guide the healing process (3).

Proliferation Phase is a process that starts on the 2<sup>nd</sup> day after the injury and continues for 3 weeks. At this stage, a basically permeable barrier is created. Epithelialization and contraction develop (4).

In response to cytokines and growth factors released from inflammatory cells in the wound area, fibroblasts begin to synthesize new extracellular matrix and immature Type III collagen. Epithelial cells originating from the basal layer at the edges of the wound create a new surface on the wound.

The remodeling phase starts in the 3<sup>rd</sup> week after the proliferation phase. At this stage, the number of fibroblasts in the wound area decreases. Collagen production reaches equilibrium and epithelialization is completed. (5).

Especially thrombocytes stimulate angiogenesis by secreting transforming growth factor beta (TGF- $\beta$ ), platelet derived growth factor (PDGF), interleukin 1 (IL-1), platelet activated growth factor (PAF), transforming growth factor alfa (TGF $\alpha$ ), tumor nekroz factor (TNF $\alpha$ ), fibroblast growth factor (FGF), epidermal growth faktor (EGF) (6).

In order to increase these effects, the use of platelet-enriched plasma-rich platelet (PRP) and plasma-rich fibrin (PRF) or the collection and injection of these directly activated factors are techniques used to accelerate wound healing (7).

Growth factors in platelet cells provide healing. Platelet cells injected rupture when they encounter calcium in the body. The Growth Factors in it repair the damaged tissues in the injected area. However, not all Platelet cells have a chance to encounter Calcium. In fact, this is the feature that distinguishes CGF from PRP. CGF is obtained by separating minimum 97% of the growth factors from the Platelet and bringing it to high density (8).

Concentrated growth factor has the isolation of a fibrin matrix denser in terms of growth factors compared to PRP and PRF (9). Therefore, CGF can be expected to have regenerative potential and better properties for clinical manipulation (10). CGF and PRF contain almost the same components; however, the high tensile strength and viscosity of CGF protect growth factors better than proteolysis (11).

Another method for wound healing is MSC injection. In theory, cells that are not limited in their ability to reproduce and renew themselves and transform into any cell are defined as stem cells (12).

While embryonic stem cells can be obtained from early blastocysts, adult stem cells can also be obtained from non-embryonic tissues. These are cord blood, hematopoietic stem cells, fat and skin cells (13).

There are local and systemic factors affecting wound healing. Factors such as blood flow in the area of the wound, cytokines and growth factors, genetic and immunological disorders, diabetes, infection, radiotherapy, chemotherapy, inappropriate nutrition, steroid drug use affect wound healing (14)

Microvascular disorder, which is one of the important complications of diabetes, neuropathy causing loss of sensation in the skin, and weakening of the ability to fight infection are the main factors that delay wound healing (15).

An excisional wound model is used in DM-induced rats to observe wound healing. An open wound is created and the time-dependent closure rate of the wound is recorded. Granulation formation, collagen deposition,

reepithelization and constriction can be investigated with this model (16).

Our aim in this study is to compare the effectiveness of CGF and PLA on wound healing in the excisional wound model created in diabetic rats.

## MATERIAL AND METHOD

A total of 30 male Wistar albino rats weighing between 300-350 g were used in this study. Experimental animals were obtained from Kırıkkale University Hüseyin Aytemiz Experimental Research and Application Laboratory. The experiment was carried out in accordance with the principles of "Guide for the Care and Use of Laboratory Animals". Approval was obtained from Kırıkkale University Animal Experiments Local Ethics Committee for the study. (Date: 02.05.2016/Issue: 16/54).

**Diabetic Rat:** Rats were carried out diabetic using Streptozotocin (STZ, Sigma Mo, USA) 55 mg/kg intraperitoneally (9). It was confirmed that morning fasting sugars were higher than 250 mg/dL with blood taken from the tail 1 week after the injection. Three groups were formed with 30 rats at 12 weeks of age. Groups consisted of 10 male rattan. One of the groups was given CGF (CGF group), the other was PLA (PLA group). Group 3 was the control group (Control group).

**Wound:** A full-thickness skin wound was created on the legs of all rats under anesthesia with xylazine HCl (1 mg/kg i.m.) + ketamine HCl (50 mg/kg i.m.) using a 5 mm punch under sterile conditions. The diameters were recorded by examining the wound areas on the 0, 3, 5 and 10 days after treatment.

**Treatment Method:** 1 day later, mesenchymal stem cells (MSC) were applied to PLA group, CGF was applied to CGF group and no medication was given to control group.

**CGF preparation:** Commercial kit (Truecell<sup>®</sup>) was used. The kit consists of two tubes with citrate as anticoagulant substance in A-tube and calcium chloride in tube B for platelet activation. 4 ml of blood taken from a rat was put into A-tube and centrifuged at 2500 rpm/min for 10 minutes. BuffyCoat layer containing dense thrombocytes and leukocytes on the surface and serum plasma part were transferred from A-tube to B-tube. It was centrifuged for 5 minutes at 4000 rpm/min. 2 ml CGF was collected, which was released from activated platelets, passed into plasma and accumulated on the surface. Then, 0.2 ml was injected into the wound area of each rat.

**PLA Preparation:** Stem cells obtained from rat adipose tissue were prepared as  $5 \times 10^6$  cells/ml. It was supplied under cold chain conditions. (Live laboratories Hospital, Istanbul, Turkey). Lipoaspirate was washed with buffer

solutions (PBS: phosphate buffer solution was used for this purpose). Enzymatic destruction was performed with collagenase. The cell layer was obtained by separating the supernatant layer by centrifugation. It was carried out by cell culture and passing after this step to obtain PLA alone.

**Sacrificion:** On the 12<sup>th</sup> day, subjects were sacrificed and blood samples were taken.

**ELISA:** TNF- $\alpha$ , TGF- $\beta$ , IL-1, PDGF, FGF and VEGF levels were measured from the blood samples taken by ELISA method.

During the experiments, five rats per cage were followed up. Maintained under standard environmental conditions (12-hour light/dark cycle, temperature ~ 21°C). It was fed ad libitum with standard rat chow and water.

**Statistical Analysis**

SPSS version 20.0 (SPSS; Chicago, IL, USA) software was used for statistical analysis. Normally distributed data were given as means $\pm$ standard deviation and non-normally distributed data as mean $\pm$ 25%. Chi-square and Fisher’s exact tests were used to compare categorical variables. Mann-Whitney U-test (MWU) and Kruskal-Wallis test (Bonferroni-adjusted) were used to compare continuous data with non-normal distributions. A p value of <0.05 was considered statistically significant.

**RESULTS**

The study was conducted on 30 diabetic rats. The rats were divided into 3 groups and there were 10 rats in each group (n=10). A full thickness wound was created with a 5 mm punch. PLA was given to the wound in group 1. CGF was given to the wound in group 2. Group 3 was the control group. Wound diameters were measured and recorded on the 3<sup>rd</sup> day, 5<sup>th</sup> day and 10<sup>th</sup> day. Blood samples were taken on the 12<sup>th</sup> day and the rats were sacrificed. TNF- $\alpha$ , TGF- $\beta$ , IL-1, PDGF, FGF and VEGF levels were measured to show the severity of the inflammatory process. Blood levels of inflammatory markers were higher than normal. However, there was no significant difference between the groups (p>0.05). The data are shown in **Table 1**.

n = 10	CGF group	PLA group	Control group	P
TNF- $\alpha$ (pg/ml)	13.4 $\pm$ 1.4	14.3 $\pm$ 2.6	13.1 $\pm$ 1.5	0.369
TGF- $\beta$ (ng/ml)	20.1 $\pm$ 2.9	18.7 $\pm$ 3.7	20.8 $\pm$ 3.8	0.481
IL-1 (pg/ml)	0.8 $\pm$ 0.2	1.0 $\pm$ 0.3	0.90 $\pm$ 0.2	0.122
PDGF (pg/mL)	39.8 $\pm$ 4.7	37.9 $\pm$ 6.7	42.47 $\pm$ 4.9	0.21
FGF (ng/mL)	17.8 $\pm$ 4.7	17.6 $\pm$ 3.4	15.5 $\pm$ 3.6	0.202
VEGF (pg/ml)	201.1 $\pm$ 4.7	198.9 $\pm$ 8.6	197.7 $\pm$ 6.3	0.525

Abbreviations: CGF: Concentrated growth factor, PLA: Processed lipoaspirate, PDGF: Platelet-derived growth factor, TGF- $\beta$ : Transforming growth factor, TNF- $\alpha$ : Tumor necrosis factor, VEGF: Vascular endothelial growth factor, FGF: Fibroblast growth factor, IL-1: Interleukin 1

The mean wound diameters measured on the 3<sup>rd</sup> day were 4.6 $\pm$ 0.06 mm in the control group, 4.1 $\pm$ 0.05 mm in the CGF group, and 4.4 $\pm$ 0.07 mm in the PLA group. Wound diameters measured on the 5<sup>th</sup> day were 3.1 $\pm$ 0.04 mm in the control group, 1.6 $\pm$ 0.05 mm in the CGF group, and 2.7 $\pm$ 0.06 mm in the PLA group. There was a significant difference in wound diameters measured on both days between CGF and PLA and control group (p<0.01). In addition, a significant difference was found between CGF and PLA groups (p<0.01). The fastest improvement was in the PLA group. The data are shown in **Table 2** and **Figure 1**.

n=10	3 <sup>rd</sup> day (mm)	5 <sup>th</sup> day (mm)	P
CGF group	4.1 $\pm$ 0.06	1.6 $\pm$ 0.004	<0.001
PLA group	4.4 $\pm$ 0.05	2.7 $\pm$ 0.05	<0.001
Control group	4.6 $\pm$ 0.07	3.1 $\pm$ 0.06	<0.001

Abbreviations: CGF: Concentrated growth factor, PLA: Processed lipoaspirate

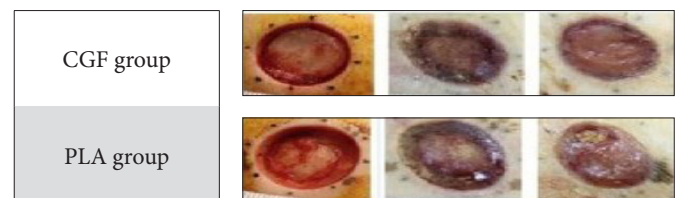


Figure 1. Wound healing status on the first, 5<sup>th</sup> and 7<sup>th</sup> days

The mean closure time of wounds was 5.3 $\pm$ 0.32 days in the CGF group, 7.1 $\pm$ 0.51 days in the PLA group, and 9.4 $\pm$ 0.4 days in the control group. A significant difference was found between the mean healing time of wounds, CGF and PLA and the control group (p<0.01). There was also a significant difference between CGF and PLA groups (p<0.01). It was observed that the fastest closure was in the CGF group and the slowest closure was in the control group. In the 10-day follow-up period, the wounds on the legs of all rats made diabetic healed. The data are shown in **Table 3** and **Figure 2**.

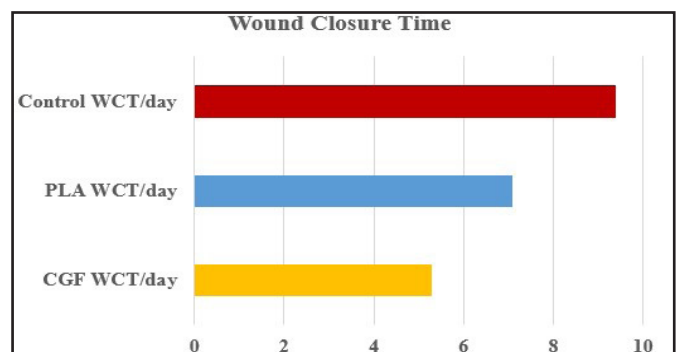


Figure 2. Wound closure time (WCT)

n=10	CGF group	PLA group	Control group	p
WCT (mean $\pm$ std)	5.3 $\pm$ 0.32	7.10 $\pm$ 0.51	9.4 $\pm$ 0.4	<0.01

Abbreviations: WCT: Wound closure time, CGF: Concentrated growth factor, PLA: Processed lipoaspirate

## DISCUSSION

Wounds can be acute (surgery, burns, penetrating injuries) or chronic (pressure sores, venous stasis ulcers, diabetic wounds, ischemic wounds, etc.). Chronic wounds are an important health problem affecting a significant portion of the population in developed countries and impairing the quality of life. In addition, its treatment brings a serious financial burden (17). Wound healing is the process of restoring the anatomical and functional properties of the tissue by regularly completing certain wound healing phases. It is known that various factors affect the wound healing process negatively. These; malnutrition, infections, diabetes, hypoxia, circulatory disorder, immunosuppression, aging and chronic diseases (18).

In vivo wound models are incisional, excisional, burn and frozen models. Models applied for cases where wound healing is impaired are malnutrition, ischemia, infection, compression and diabetes models. In this experiment, we investigated wound healing in diabetic rats using an excisional wound model (19).

It has been shown that a significant part of the proliferation, migration and vascular formation promoting effects of PRP are achieved through exosome-like molecules released from platelets into the plasma (20). The effect of PRP on wound healing has been mainly associated with growth factors released from platelets (21). CGF is more intense in terms of growth factors compared to PRP and PRF (22). In our study, instead of PRP, growth factors (GF) released from leukocyte-free and activated PRPs were applied to the wound area.

The most important inflammation cytokines in wound healing are TNF- $\alpha$ , TGF- $\beta$ , IL-1, PDGF, FGF and VEGF. (23). Blood levels in the groups were measured by ELISA method. All were found higher than normal levels. But there was no statistically significant difference between them ( $p > 0.05$ ). The similar inflammatory mediator levels were interpreted as similar rates of local and systemic inflammation in the treatment groups. The high levels were interpreted as tissue repair and healing continued. It has been shown that the proliferation phase, which is the last phase of wound healing, can continue for up to 6 weeks (24). Towards the end of this process, it was thought that there might be a differentiation between the treatment group and the treatment group.

In wound healing, epithelial cells migrate from the beginning of the injury until the entire damaged surface is covered. Wound contraction begins to occur 7 days after injury, and myofibroblasts play an important role at this stage. Many factors affect the healing and contraction process at a rate of approximately 0.75 mm/day (5-7). In this study, the mean wound diameters on the 3<sup>rd</sup> day

were  $4.6 \pm 0.06$  mm in the control group,  $4.1 \pm 0.05$  mm in the CGF group, and  $4.4 \pm 0.07$  mm in the PLA group. Wound diameters measured on Day 5 were  $3.1 \pm 0.04$  mm in the control group,  $1.6 \pm 0.05$  mm in the CGF group, and  $2.7 \pm 0.06$  mm in the PLA group ( $p < 0.01$ ). These results show that both PLA and CGF increase wound repair in diabetic rats compared to the control group. The fastest recovery seems to be in the CGF group.

In an animal study where the reconstruction of bone defects was evaluated using CGF, PRP and PRF, they were compared in terms of their osteogenic potential, but no statistically significant difference was found between them (25). First developed by Sacco (26) in 2006, CGF, one of the second-generation platelet concentrations, demonstrated the potential to accelerate osteogenesis when used in sinus augmentation.

In the rat calvarial bone defect regeneration study of Khojasteh et al. (27) it was reported that MSC application yielded more successful results than PRP.

It has been shown in different studies that stem cell application will contribute to the treatment of difficult-to-heal wounds such as diabetic ulcers (28).

Walter et al. (20) MSCs associated their contribution to wound healing with the chemotactic mediators they secrete, such as TGF-1 $\beta$ , IL-6, and IL-8. The clinical benefits of MSCs can be summarized as stimulation of cellular repair, attenuation of inflammation, enhancement of angiogenesis and therapeutic cell migration (29).

The mean closure time of the wounds in the study was  $5.3 \pm 0.32$  days in the CGF group,  $7.1 \pm 0.51$  days in the PLA group, and  $9.4 \pm 0.4$  days in the control group. A significant difference was found between the mean healing time of wounds, CGF and PLA and the control group ( $p < 0.01$ ). There was also a significant difference between CGF and PLA groups ( $p < 0.01$ ). It was observed that the fastest closure was in the CGF group and the slowest closure was in the control group. In the 10-day follow-up period, the wounds on the legs of all rats made diabetic healed. These results in diabetic rats have shown that CGF and PLA are effective in wound healing as in other studies. In the study, it was found that CGF is more effective than PLA. We think that the reason for CGF's effectiveness is its active and long effect.

## CONCLUSION

In this study, CGF and PLA applications are two important methods that increase wound healing, but CGF application has been shown to be a more effective method than PLA in wound healing.

## ETHICAL DECLARATIONS

**Ethics Committee Approval:** Approval was obtained from Kırıkkale University Animal Experiments Local Ethics Committee for the study. (Date: 02.05.2016/Issue: 16/54).

**Referee Evaluation Process:** External double-blind referee assessment.

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**Author Contributions:** All of the authors declare that they have all participated in the design, execution, and analysis of the paper, and that they have approved the final version.

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